IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	Christine Markert-Hahn *et al.*
Application No.	:	10/647,720
Filed	:	August 25, 2003
For	:	METHOD FOR BISULFITE TREATMENT

Examiner	:	Joyce Tung
Art Unit	:	1637
Docket No.	:	810102.401

DECLARATION OF MATTHIAS BALLHAUSE
PURSUANT TO 37 C.F.R. §1.132

I, Matthias Ballhause, declare as follows:

1. I currently hold the position of Scientist at Epigenomics AG, located in Berlin, Germany, a company affiliated with the assignee of the above-identified application (the "Application").

2. As evident from the enclosed CV, I have been involved in biochemical research since I commenced my chemistry studies at the Technical University of Berlin in 1997. I joined Epigenomics AG in February 1999, and in the field of DNA methylation I have been involved in as part of my responsibilities:

- Light-directed DNA synthesis on solid surfaces with photolabile protected DNA-monomers
- Chip-hybridization technologies
- Development and of bisulfite-conversion and purification protocols.
- Optimization of bisulfite-protocols for different sample-types (blood-plasma, blood-serum, FFPE) regarding complete bisulfite-conversion and purification.
- Evaluation of new post-bisulfite purification technologies with focus on ultrafiltration and DNA-binding to silica surfaces (spin-columns, magnetic particles).
- Optimization of DNA-extraction methods from various sample-types (blood-plasma, blood-serum, FFPE) with new technologies. The focus was set on

methods which can be performed on a liquid-handling robot. Therefore magnetic beads were evaluated.

- Influence of residuals from bisulfite-purification on PCR performance of real-time PCR assays.

Several publications in peer-reviewed scientific journals verify my expertise in the field of DNA methylation. I am also an inventor on a number of patents and patent applications (see enclosed list of published patent applications).

3. It is fair to say that I have profound knowledge not only of the bisulfite method and DNA methylation based diagnostic technology described in the Application, but of the knowledge available in the field as of the time of filing the Application.

4. I submit this Declaration as evidence of the non-obviousness of the presently claimed bisulfite reaction-based method over the prior art references cited in the Office Actions issued during the examination proceedings of the Application. I am familiar with the Application and have reviewed the outstanding final Office Action mailed on August 12, 2009, in addition to the documents referred to by the Examiner in this Office Action. I provide my opinion on the invention as defined in the claims currently pending, taking into account the disclosure of the Application as originally filed, the general knowledge available in the art at the time the Application was filed, and the teachings from the below referenced prior art.

5. The present invention relates to an improved bisulfite reaction-based method for the conversion of cytosine bases in a nucleic acid to uracil bases. The presently claimed method is useful for determining methylation positions in a nucleic acid which, in turn, is of interest for diagnostic purposes in the field of epigenomics. The method is based, in pertinent part, on performing the deamination step, the desulfonation step or both on a solid phase-bound nucleic acid, such as a patient DNA.

6. I confirm herewith the statement made by Christine Markert-Hahn in her Declaration dated April 11, 2008, namely that at the time of filing the Application, a person skilled in the art would not have reasonably expected that a denatured nucleic acid bound to a solid phase, such as a magnetic glass particle or silica surface, could have been subjected to bisulfite treatment, as claimed in the Application. When performing a conventional bisulfite reaction at the time of filing the Application, DNA was denatured since bisulfite ions were believed to react only with pyrimidine bases like cytosine which are not involved in base-pairing. If, however, DNA is bound to a solid

phase like glass, a number of interactions of DNA and the solid phase will take place. Accordingly, I confirm that at the time of filing the Application it was generally known in the art that the binding of single-stranded DNA to a solid phase involves various interactions, including hydrogen bonds. In 2003, a person skilled in the art understood that the interactions between a single-stranded DNA molecule and a solid phase were similar to the interactions between two individual strands in a double-stranded DNA molecule. Since the conventional knowledge in the art had suggested that binding DNA to a solid phase will result in a nucleic acid behaving in a manner similar to double-stranded nucleic acids, it was the common view that solid phase-bound DNA will hence not be susceptible to bisulfite modification.

7. The above-described common view finds its expression also in the prior art: Hayatsu 1997 (copy enclosed) describes at page 1365, right column, 2nd full paragraph, that bisulfite is a single-strand specific, cytosine deaminating agent, and that the single-strand specific nature of this chemical reaction was retained for the DNA complexed with chitosan. Likewise, Olek 1996 (cited in the examination proceedings of the Application) describes that the bisulfite treatment was performed under conditions maintaining the DNA in the single stranded form so as to ensure an optimal bisulfite reactivity (*see* Abstract of Olek 1996).

8. Since bisulfite reacts only with unmethylated cytosines that do not participate in base pairing (*i.e.*, single stranded DNA), a person skilled in the art would have been surprised to learn that a bisulfite reaction could be accomplished using a single stranded DNA bound to a solid phase. There is no teaching in the cited art that would have changed a skilled person's surprise in this matter. Thus, it was a surprising and unexpected finding when the inventors of the Application found out that single stranded DNA bound to a solid phase could actually be accessed successfully by bisulfite ions. This unexpected finding allows the process to be automated and renders the results obtained by the claimed method much more precise and accurate than was known in the art before the time of filing the Application.

9. In summary, I submit that, at the time the Application was filed, it would not have been obvious to a skilled worker, who had knowledge of the cited art, to use solid phase bound denatured DNA for bisulfite treatment, as defined in the claims.

10. I hereby declare that all statements made herein are, to my own knowledge, true and that all statements made on information or belief are believed to be true; and further

that these statements are made with the knowledge that wilful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such wilful false statements may jeopardize the validity of the captioned patent application or any patent issued therefrom.

Date 02.02.2010

Matthias Ballhause

Encls.:
Curriculum Vitae
List of published patent applications
Hayatsu et al. 1997 (Chem. Pharm. Bull **45**(8):1363-1368)

EPI - Ref. Number	Patent / Registration No.	application no.	title	date of publication	publication number	Inventor
P084AU_RA	2002363259	2002-363259	Method for the Detection of Cytosine Methylations in immobilized DNA samples	12.05.03	AU 2002363259	K.Berlin, M.Ballhause, D.Gütig
P084DE_RA	101 54 317	10154317.4	Verfahren zum Nachweis von Cytosin-Methylierung in immobilisierten DNA Proben	15.05.03	DE 101 54 317	K.Berlin, M.Ballhause, D.Gütig
P084EP_RA	1436437	02802274.7	Method for the Detection of Cytosine Methylations in immobilized DNA samples	21.07.04	EP 1436437	K.Berlin, M.Ballhause, D.Gütig
P084EP-1_RA		09157992.0	Method for the Detection of Cytosine Methylations in immobilized DNA samples	05.08.09	2085487	K.Berlin, M.Ballhause, D.Gütig
P084JP_RA		2003-540386	Method for the Detection of Cytosine Methylations in immobilized DNA samples	10.03.05	JP 2005-506850	K.Berlin, M.Ballhause, D.Gütig
P084US_RA	7,407,749	10/416,824	Method for the Detection of Cytosine Methylations in immobilized DNA samples	17.06.04	US 2004-0115663	K.Berlin, M.Ballhause, D.Gütig
P084US-1_RA	7,534,570	11/981,357	Method for the Detection of Cytosine Methylations in immobilized DNA samples	15.05.08	US 2008-0113379	K.Berlin, M.Ballhause, D.Gütig
P134DE_AD	103 47 397	103 47 397.1	Optimierte Bisulfit-Umwandlung durch Zusatz von n-Alkylenglykol-Verbindung	19.05.05	DE 103 47 397	K.Berlin, M.Ballhause
P135DE_AD	103 47 400	103 47 400.5	Verbesserte Bisulfit-Umwandlung von DNA durch kurzzeitige Temperaturerhöhung	19.05.05	DE 103 47 400	M.Ballhause
P178EP_AD		05802810.5	Method for providing DNA Fragments derived from an archived sample	13.06.07	EP 1794319	K.Berlin, M.Ballhause, D.Dietrich, A.Kluth, M.Schuster, U.Wagner, R.Wasserkort, H.Zebarth
P178US_AD		11/684,387	Method for providing DNA Fragments derived from an archived sample	11.09.08	US-2008-0220418-A1	K.Berlin, M.Ballhause, D.Dietrich, A.Kluth, M.Schuster, U.Wagner, R.Wasserkort, H.Zebarth
P179AU_RA	2004282342	2004282342	Improved bisulfite conversion of DNA	28.04.05	AU 2004282342	K.Berlin, K.Cardon, M.Ballhause
P179CA_RA		2,540,310	Improved bisulfite conversion of DNA	28.04.05	CA 2540310	K.Berlin, K.Cardon, M.Ballhause
P179CN_RA		200480029442.3	Improved bisulfite conversion of DNA	01.12.06	CN 100037	K.Berlin, K.Cardon, M.Ballhause
P179EP_RA	1670949	04790548.4	Improved bisulfite conversion of DNA	28.04.05	EP 1670949	K.Berlin, K.Cardon, M.Ballhause
P179EP-1_RA		EP09159859.9	Improved bisulfite conversion of DNA	12.08.09	2088210	K.Berlin, K.Cardon, M.Ballhause
P179JP_RA		2006-530157	Improved bisulfite conversion of DNA	05.04.07	JP 2007-506007	K.Berlin, K.Cardon, M.Ballhause
P182US-1_AD		12/410,389	Improved bisulfite conversion of DNA	22.10.2009 22.06.2006	US-2009-0263810 US 2006-0134643	K.Berlin, K.Cardon, M.Ballhause
P200EP_AD		06742549.6	Improved method for bisulfite conversion	26.12.07	EP 1869215	I.Schwope, M.Ballhause M.Ballhause, K.Berlin, Th. deVos, D.Dietrich, V.Liebenberg, C.Lofton-Day, J.Lograsso, J.Maas, F.Model, M.Schuster, A.Sledziewski, R.Tetzner
P246EP_AD		06750657.6	Method for providing DNA Fragments derived from a remote sample	02.01.08	EP 1871912	M.Ballhause, K.Berlin, Th. deVos, D.Dietrich, V.Liebenberg, C.Lofton-Day, J.Lograsso, J.Maas, F.Model, M.Schuster, A.Sledziewski,
P246JP_AD		2008-506828	Method for providing DNA Fragments derived from a remote sample	02.10.08	2008-537888	

Curriculum Vitae

Name:	Matthias Ballhause
Date of Birth:	January, 18th 1969
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Profession:	Analytical Chemist

Education

1975 – 1981	Primary School: Rudolf-Hildebrand Grundschule Berlin
1981 – 1988	Highschool: Luise-Henriette Gymnasium Berlin
1988 – 1997	Chemistry Studies: Technical University Berlin
April 1997 – March 1998	Diploma Thesis 'Application of analytical methods for detecting phosphorous compounds in suspended matter of the River Spree' Leibnitz Institute of Freshwater Ecology and Inland Fisheries Müggelseedamm 301 12587 Berlin / Germany

Professional Career

January 1998 – August 1998	Mobility of Phosphorous in Sediments of Shallow-Lakes, Renaturation of eutrophic Lakes Leibnitz Institute of Freshwater Ecology and Inland Fisheries Müggelseedamm 301 12587 Berlin / Germany
September 1998 – February 1999	Job Search
February 1999 –	Epigenomics AG Kleine Präsidentenstrasse 1 10178 Berlin / Germany